Embryo collection in clone cattle offspring

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EMBRYO COLLECTION IN CLONE CATTLE OFFSPRING

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Our laboratory has been working on bovine clones for many years. These clones were studied and several cloned females were bred to obtain clone offsprings. All pregnancies were normal and calves developed as healthy individuals. The females were used for embryo collection after superovulation. The objective of this study was to compare the embryo recovery results between clone offspring and control animals. Altogether, 28 cows were used for this study (18 clone offspring and 10 controls). All the animals were born and raised in the same experimental farm, in the same time period and in the same rearing conditions. 90 flushes were performed to collect D9 to D21 embryos for research protocols. For early embryos on D9, a classical 3 way collection equipment (IMV, France) was used. To collect the late embryos D12-D21, the same equipment was modified so that larger embryos could be collected through the remaining larger hole (2 way collection) (Richard et al. 2015, Theriogenology 83,1101-9). All females were submitted to ovum pick-up to remove the dominant follicle and were subsequently superovulated with FSH (Stimufol®, Reprobiol, Belgium). Luteolysis was induced 48 hours prior to AI. Two AI were performed with frozen semen, 48 and 56 hours after PGF2□ injection (Estrumate®, MSD Santé Animale, France). Before embryo collection, cows were treated with an epidural injection of 3-4 ml (Xylovet®, CEVA Santé Animale SA, France).The presence of Copora Lutea (CL) was checked and they were counted by rectal palpation. For all collections, the cervix was prepared with the initial introduction of a dilator. Then the catheter was introduced in one horn and the cuff was inflated as low as possible. For the collection of late stage embryos, 30 ml (Euroflush, IMV, France) was injected slowly twice to suspend the embryos prior to flushing the horn with 500 ml, and the same operation was performed on the second horn. Data were analyzed by unpaired t-test using Prim® software. There was no significant difference in the number of embryos collected per flush in clone offspring and controls (349 embryos collected, 5.05 ± 4.8 per flush vs 90 embryos collected, 4.28 ± 3.92 per flush, respectively). The number of CL was also not significantly different between groups (11.49 ± 7.32 and 8.43 ± 4.26 per flush, respectively). For late collections in all animals, the FSH dose (Stimufol®) was reduced to limit the number of embryos and preserve development (Richard et al. 2015). Retrospectively there was no significant difference for the necessary dose for superovulation (0.57 ± 0.08 for clone offspring and 0.54 ± 0.07 for controls). These data indicate that offspring of clones raised since birth in the same conditions as control heifers have the same ability to give embryos after superovulation treatment indicating equivalence of reproductive function.